TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		Attorney's Docket Number 045636-5051
		U.S. Application No. Unassigned 09/936697
International Application. No.	International Filing Date	Priority Date Claimed
PCT/FR00/00613	March 14, 2000	March 15, 1999

Title of Invention

GRB 14 AND THE INSULIN RECEPTOR AND SCREENING OF NOVEL MEDICINES

Applicants For DO/EO/US

Anne-Francoise BURNOL, Dominique PERDEREAU, Anne Kasus-JACOBI, Veronique BEREZIAT and Jean GRARD

Applicants herewith submit to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [X] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [X] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [x] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [x] An oath or declaration of the inventors (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 14. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [x] Other items or information:
    a. [x] WO 00/556344
    b. [ ] PCT/IB/304
    c. [ ] PCT/IB/308
    d. [ ] PCT/IPEA/409
    e. [ ] Paper Copy of Sequence Listing
    f. [ ] Diskette with Sequence Listing in C.R.F.
    g. [ ] Statement Accompanying Sequence Listings

U.S. APPLICATION NO.	INTERNATIONAL APPLICATION NO.	ATTORNEY DOCKET NUMBER
Unassigned 09/936497	PCT/FR99/00109	531 Rec'd PCT/ 17 SEP 2001 045636-5088

15. [X] The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)-(5)):

Search Report has been prepared by the EPO or JPO.....$860.00
International preliminary examination fee paid to USPTO (37 CFR 1.482).....$690.00
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....$710.00
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$1000.00
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....$100.00

ENTER APPROPRIATE BASIC FEE AMOUNT = $ 860.00

Surcharge of $130.00 for furnishing the oath or declaration later than [ ] **20** [ ] **30** months from the earliest claimed priority date (37 CFR 1.492(e)). $

Claims	Number Filed	Number Extra	Rate	
Total Claims	20 - 20 =	0	X $18.00	$
Independent Claims	4 - 3 =	1	X $80.00	$ 0.00
Multiple dependent claim(s) (if applicable)			+$270.00	$ 0.00

TOTAL OF ABOVE CALCULATIONS = $

Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28) -$

SUBTOTAL = $ **860.00**

Processing fee of $130.00 for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +$

TOTAL NATIONAL FEE = **$860.00**

Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +$

TOTAL FEES ENCLOSED = $ **860.00**

Amount to be refunded $

Amount charged $

a. [ ] A check in the amount of $-0- to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 50-0310 for **$860.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] **Except** for issue fees payable under 37 C.F.R. §1.18, the Commissioner is hereby authorized by this paper to charge any additional fees during the entire pendency of this application including fees due under 37 CFR §1.16 and §1.17 which may be required, or credit any overpayment to Deposit Account No. 50-0310.

Customer No. 009629
SEND ALL CORRESPONDENCE TO:
Morgan, Lewis & Bockius LLP
1800 M Street, N.W.
Washington, D.C. 20036
(202) 467-7000

Elizabeth C. Weimar

Elizabeth C. Weimar
Reg. No. 44,478

Submitted: September 17, 2001

PATENT
ATTORNEY DOCKET NO.: 045636-5051

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Anne-Francoise BURNOL, et al.

U.S. National Phase Application
Filed: September 17, 2001

U.S. Application No.: To Be Assigned

Date of National
Stage Entry: Concurrently — Art Unit: Unassigned

Based on PCT/FR00/00613 — Examiner: Unassigned

Filed: March 14, 2000

For: GRB 14 AND THE INSULIN RECEPTOR AND SCREENING OF NOVEL MEDICINES

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Prior to examination in the above-identified application please enter the following amendments:

IN THE CLAIMS:

Please cancel claims 1-7 and add the following claims 8-20.

8. A fragment consisting of the PIR domain or the PIR-SH2 domain of any one of the proteins in the Grb7 family of proteins.

9. The fragment of claim 8 selected from the group consisting of any one of the peptide sequences of SEQ ID NO: 1 to SEQ ID NO: 28.

10. A method for detecting molecules capable of modulating the tyrosine kinase activity of the insulin receptor, comprising:

a) bringing an activated insulin receptor into contact with a fragment consisting of the PIR domain or the PIR-SH2 domain of any one of the proteins in the Grb7 family of proteins, and the molecule to be tested, under conditions which allow binding of said fragment to said receptor,

b) adding a tyrosine kinase substrate,

c) measuring the tyrosine kinase activity, and

d) determining the modulation of the tyrosine kinase activity by comparison with a control consisting of the activated insulin receptor and said fragment.

11. The method of claim 10, wherein said fragment is selected from the group consisting of any one of the peptides of SEQ ID NO: 1 to SEQ ID NO: 28.

12. The method of claim 10 further comprising preselection prior to step a) wherein molecules capable of modulating the interactions of a fragment consisting of the PIR domain or the PIR-SH2 domain of any one of the proteins in the Grb7 family of proteins with the insulin receptor are identified, said preselection comprising:

1) immobilizing said fragment on a solid support,

2) bringing the molecule to be tested into contact with said fragment, then

3) incubating with a labeled and pre-activated insulin receptor, under conditions which allow binding of said receptor to said fragment,

4) separating said labeled receptor not retained on the support,

5) detecting the complex possibly formed between said fragment and said activated insulin receptor, and

6) determining the effect of the molecule by comparison with a control comprising said fragment and said insulin receptor absent the molecule to be detected.

13. The method of claim 12 wherein the fragment is selected from the group consisting of any one of the peptides of SEQ ID NO: 1 to SEQ ID NO: 28.

14. A method of treating a disease involving insulin comprising the administration of an effective amount of a molecule capable of binding to a fragment consisting of the PIR domain or the PIR-SH2 domain of any one of the proteins in the Grb7 family of proteins and of inhibiting the tyrosine kinase activity of the insulin receptor.

15. The method of claim 14, wherein said molecule is identified using the method of claim 10.

16. The method of claim 14, wherein said molecule is identified using the method of claim 11.

17. The method of claim 14, wherein said molecule is identified using the method of claim 12.

18. The method of claim 14, wherein said molecule is identified using the method of claim 13.

19. The method of claim 14, wherein said disease involving insulin is obesity.

20. The method of claim 14, wherein said disease involving insulin is diabetes.

REMARKS

Applicants respectfully submit that no prohibited new matter has been introduced by this Preliminary Amendment and that claims 18 to 20 are drawn to the same invention as claims 1-7 of International Application PCT/FR00/00613. The changes to the claims represent changes in formalities so as to bring the claims into compliance with the rules of practice in the United States, such as: "use" claims are not a recognized category of invention (see original claims 2, 6 and 7); to provide established claim terminology to describe the intended scope of the claims, i.e. incorporation of the terms "comprising" and "wherein" rather than "containing" and "characterized in that" (see claims 1-7); to avoid improper multiple dependency (see claims 5 and 7) and to correct grammar such as noun placement and tense (see all of the original claims). In addition, claims 19 and 20 have been added to include two specific embodiments of the contemplated treatment methods. Support for these embodiments may be found throughout the specification and particularly on page 5, lines 16-34.

If there are any additional fees due in connection with the filing of this Preliminary Amendment, please charge the fees to our Deposit Account No. 50-0310.

Respectfully submitted
MORGAN, LEWIS & BOCKIUS LLP

Elizabeth C. Weimar

Elizabeth C. Weimar
Reg. No. 44,478

Dated: September 17, 2001
MORGAN, LEWIS & BOCKIUS LLP
1800 M Street, N.W.
Washington, DC 20036
202-467-7812

GRB14 AND THE INSULIN RECEPTOR AND ALSO SCREENING FOR NOVEL MEDICINAL PRODUCTS

The present invention relates to the use of the Grb14 protein and of homologous adapter proteins (proteins of the Grb7 family), as a tool for screening for molecules intended for treating diseases involving insulin.

Insulin, which is the principal hormone for the regulation of energy metabolism, is the only blood-glucose-lowering hormone in the body; it stimulates the transport of glucose and its use by peripheral tissues (skeletal muscles and adipose tissue) and inhibits the endogenous production of glucose by the liver.

Insulin acts through a receptor which is expressed at the plasma membrane of cells. This receptor is part of the family of receptors with tyrosine kinase activity, which are characterized by the presence of an intracellular domain which bears the catalytic activity. Binding of the ligand induces dimerization of the receptors, activation of the tyrosine kinase domain and phosphorylation (autoposphorylation and transphosphorylation) of specific tyrosine residues present in the cytosolic component of the receptors (Ullrich. A. et al. (1990) *Cell*, **61**, 203-212).

The insulin receptor has the particularity of being present in a naturally dimerized form. The binding of the insulin to the extracellular α subunit induces conformation modifications which result in the activation of the kinase domain borne by the β subunit of the receptor, and in its autophosphorylation, which is required for complete activation of the receptor. The insulin receptor activated in this way phosphorylates intracellular proteins which are used as insulin signal effectors.

Specifically, the transduction of a signal inside the cell, after a receptor with tyrosine kinase activity has been stimulated, makes use of protein-protein interaction cascades which result in a metabolic or mitogenic effect, and in which the molecular adapters have a preferred role. Through their protein interaction domains, the adapters enable the recruitment of successive effectors, constituting the signalling pathways.

Among the various relays between the insulin receptor and its intracellular effectors, the most well characterized adapter proteins are IRS-1, IRS-2 (*Insulin Receptor substrate-1 and 2)* and Shc (*Src and collagen homologous protein)* (White M.F. et al. (1994) *J. Biol. Chem.*, **269**, 1-4; Waters S.B., et al. (1996) *Trends Cell Biol.*, **6**, 1-4). They are not specific for insulin-sensitive tissues and are also phosphorylated both after the activation of other tyrosine kinase receptors and after that of cytokine receptors or G protein-coupled receptors (Bonfini L. et al. (1996) *Trends Biochem. Sci.*, **21**, 257-261; Souza S.C. et al. (1994) *J. Biol. Chem.*, **269**, 30085-30088; Argetsinger L.S. et al. (1995) *J. Biol. Chem.*, **270**, 14685-14692; Platanias L.C. et al. (1996) *J. Biol. Chem.*, **271**, 278-282; Velloso L.A. et al. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 12490-12495; Kowalski-Chauvel A. et al. (1996) *J. Biol. Chem.*, **271**, 26536-26361).

Thus for example, the Shc protein binds to the activated insulin receptor, is then phosphorylated and recruits the Grb2 adapter which binds to phosphotyrosine residues of Shc, through its SH2 domain, and binds, via an SH3 domain, to the nucleotide exchanger Sos, which will itself enable the activation of Ras (Schlessinger, J. (1993), *Trends Bioch. Sci.*, **18**, 273-275).

Recently, novel adapter proteins which may be specifically involved in insulin signal transduction have been cloned by interaction with the insulin receptor using the double-hybrid system, in particular various isoforms of the Grb10 protein from humans and from mice (Liu F. et al. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 10287-10291; O'Neill T.J. et al. (1996) *J. Biol. Chem.*, **271**, 22506-22513; Frantz J.D. et al. (1997) *J. Biol. Chem.*, **272**, 2659-2667).

Even more recently, the inventors have cloned the rat rGrb14 and rGrb7 proteins by interaction with the insulin receptor using the double-hybrid system (Kasus-jacobi et al. (1998) *J. Biol. Chem.*, **273**, 26026-26035).

The mGrb10, hGrb10, hGrb14 and rGrb14 proteins belong to the same family of adapter proteins, the first known member of which is the Grb7 protein which binds to the receptor for EGF, for Ret and for PDGF (Margolis B. (1992) *Proc. Natl. Acad. Sci. USA*, **89**, 8894-8898). Hereinafter, the proteins of this family are termed proteins of the Grb7 family.

These proteins which have been cloned by interaction with activated insulin receptors appear to play an important role in insulin signal transduction.

Thus, the inventors have shown that the expression of the rGrb14 protein is very well correlated with the sensitivity of tissues to insulin and that its overexpression in CHO-IR cells (*Chinese Hamster Ovary* cells expressing high levels of insulin receptors of human origin) inhibits the effects of insulin by decreasing the activation of IRS-1 without modifying the autophosphorylation of the insulin receptor (Kasus-Jacobi et al. (1998) already cited).

The adapter proteins of the Grb7 family are characterized by the succession of three domains:

- a proline-rich sequence named PP, close to the amino-terminal end,
- a central domain named PH (*Pleckstrin homology*) and
- a domain named SH2 (*Src homology 2*) at the carboxy-terminal end, known to interact with sequences containing phosphotyrosines (Ooi J. et al. (1995) Oncogene, **10**, 1621-1630; Margolis B. (1992) already cited; Daly R.J. (1996) already cited).

Besides these domains which have already been studied in other proteins, the inventors have revealed a novel domain on the rGrb14 protein, named PIR (*Phosphorylated Insulin Receptor Interacting Region*) corresponding to residues 340 to 437 of the protein; by comparison between the Grb7, Grb10 and Grb14 proteins, the inventors have shown that a 43 amino acid sequence corresponding to amino acids 365 to 470 of the rGrb14 protein is highly conserved throughout the family of these proteins (Kasus-Jacobi et al. (1998) already cited) and should play a specific role in the attachment of these proteins to the insulin receptor.

The PIR domain is homologous to the BPS domain (*Between PH and SH2*) (Kasus-Jacobi et al. (1998) already cited), recently demonstrated on the hGrb10 protein (He W. et al. (1998) *J. Biol. Chem.*, **273**, 6860-6867), and corresponds to amino acids 358-434 of the Grb14 protein.

The association between the activated insulin receptor and the proteins of the Grb7 family involves the two domains PIR and SH2. Depending on the Grb protein under consideration, the respective role of the two domains is more or less important. Specifically, it is

essentially PIR which is responsible for the binding of Grb14 to the insulin receptor (Kasus-Jacobi et al. (1998) already cited), whereas PIR and SH2 are involved in the interaction between Grb10 and the receptor (He et al (1998) already cited).

Several teams have shown that there are defects in phosphorylation of the insulin receptor and also modifications of the effects of insulin on the transport of glucose and on the activation of certain enzymes in obese or diabetic patients (Arner, P. et al., J. N. (1987), *Diabetologia*, **30**, 437-440; Caro, J. F. et al. (1987), *J. Clin. Invest.*, **79**, 1330-1337; Mandarino, L.J. (1989), *Diab. Metab. Rev.*, **5**, 475-486).

Mutations of the insulin receptor gene may lead, via various mechanisms, to a decrease in the tyrosine kinase activity of the receptor, thus contributing to the development of a condition of insulin resistance and to the institution of pathological conditions such as obesity and non-insulin-dependent diabetes (DNID) (Taylor, S.I. (1992), *Diabetes*, **41**, 1473-1490).

In conditions of insulin resistance, hyperglycemia develops when the endogenous secretion of insulin is no longer sufficient, and it is necessary to resort to insulin therapy in order to maintain carbohydrate homeostasis. After the diabetes has evolved for 10 years, severe complications are observed in 30% of cases. These complications, which are secondary to poor control of glycemia, have various very serious clinical implications (renal failure, necrosis and amputation of the lower limbs, blindness) which lead to a shortening of the life expectancy of the patients.

Normalization of the tyrosine kinase activity, when it is disturbed, may be envisioned either directly, using molecules which act on this enzyme (Levitzki et al.

(1995), *Science* **267**, 1782-1788) or indirectly, by inhibiting the interactions between the adapter proteins and the tyrosine kinase (Pendergast et al. (1993), *Cell*, **75**, 175-185).

Now, the inventors have shown, surprisingly, that the binding, to the activated insulin receptor, of the PIR domain of the proteins of the family of Grb7 proteins (Grb14, Grb10 and Grb7), alone or associated with the SH2 fragment (PIR-SH2), inhibits the tyrosine kinase activity of said receptor.

The subject of the present invention is the use of a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, as a tool for screening for molecules intended for treating diseases involving insulin.

According to an advantageous embodiment of said use, said fragment is selected from the group consisting of the sequences: SEQ ID NO: 1-28 which correspond, respectively, to PIR fragments (residues 365-407 and residues 353-436) and to PIR-SH2 fragments (residues 365-538 and residues 353-538) of the rGrb14, hGrb14, mGrb10, hGrb10, rGrb7, hGrb7 and mGrb7 proteins.

For the purposes of the present invention, the numbering of the residues of the protein fragments is given with reference to the sequence of the rGrb14 protein after alignment.

Interestingly, the inventors have shown that the inhibitory effect of the Grb14 protein is reproduced by the purified GST-PIR and GST-PRI+SH2 fusion proteins, which are obtained by fusion of GST with the PIR domain or the PIR+SH2 domain of rGrb14. On the other hand, this inhibitory effect is not observed with the *GST-SH2*

fusion protein obtained by GST fusion with the SH2 domain of rGrb14.

Unexpectedly, the inventors have shown that the PIR domain alone has an activity equivalent to that of the whole protein, whereas the PIR+SH2 domain has a much greater inhibitory effect than PIR expressed alone. Specifically, total inhibition of the tyrosine kinase activity of the insulin receptors is obtained when 0.3 µg of GST-PIR protein is added, whereas only 0.03 µg of GST-PIR+SH2 is necessary. It appears, therefore, that, while the SH2 domain has no inhibitory activity per se, on the other hand it greatly potentiates the effect of PIR.

In comparable fashion, the inventors have shown that the PIR and PIR+SH2 domains of Grb10 have an inhibitory effect on the tyrosine kinase activity of the insulin receptor. The SH2 domain of Grb10 does not, in itself, have an inhibitory effect, but it too potentiates the inhibition induced by PIR.

In addition, the inventors have shown that the insulin receptor is more sensitive to the inhibitory effect of Grb14 than to that of Grb10 and of Grb7, and that the effect may be obtained both with the whole protein and with the PIR domain or the PIR-SH2 domain.

The PIR and PIR-SH2 domains of the Grb14, Grb10 and Grb7 proteins therefore behave like endogenous inhibitors of the tyrosine kinase activity of the insulin receptor, which is an entirely novel function for molecular adapters. In fact, unlike the adapter proteins IRS-1, IRS-2 or Shc which are intermediates between the insulin receptor and cellular effectors, said domains of the proteins of the Grb7 family act directly on the tyrosine kinase activity of the insulin receptor.

Consequently, the PIR and PIR-SH2 domains of the Grb14 protein and of the homologous adapter proteins (proteins of the Grb7 family) constitute potential targets for medicinal products.

Specifically, compounds which are capable of increasing or of suppressing the interactions of the domains of said proteins may prevent or cure disorders of the organism which are related to a modification of the activity of the kinase protein of the insulin receptor.

Consequently, the present invention therefore also relates to a method for detecting molecules capable of stimulating or inhibiting (modulating) the tyrosine kinase activity of the insulin receptor, characterized in that it comprises:

a) bringing the activated insulin receptor into contact with a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, and the molecule to be tested, under conditions which allow binding of said fragment to said receptor,

b) adding a tyrosine kinase substrate,

c) measuring the tyrosine kinase activity, and

d) determining the modulation (inhibition or stimulation) of the tyrosine kinase activity by comparison with a control consisting of the activated insulin receptor and said fragment.

In accordance with the invention, said fragment is preferably selected from the group consisting of the sequences SEQ ID NO: 1 to 28.

According to an advantageous embodiment of said method, prior to step a) above, a preselection of the molecules capable of stimulating or inhibiting (modulating) the interactions of a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, with the insulin receptor, is carried out by:

1) immobilizing said fragment on a solid support,

2) bringing the molecule to be tested into contact with said fragment, then

3) incubating with the labeled and pre-activated insulin receptor, under conditions which allow binding of said receptor to said fragment,

4) separating said labeled receptor not retained on the support,

5) detecting the complex possibly formed between said fragment and the activated insulin receptor, and

6) determining the effect of the molecule (inhibition or stimulation of the fragment-receptor interaction), by comparison with a control comprising said fragment and the insulin receptor.

In order to allow said fragment to be immobilized on a solid support, said fragment may, for example, be expressed as a fusion with a protein such as GST.

Said receptor may, for example, be labeled with a radioactive molecule or fused to a fluorescent protein such as GFP (*Green Fluorescent Protein*).

When said receptor is labeled with a fluorescent or radioactive molecule, the interaction between said fragment and said receptor is detected by reading the fluorescence or the radioactivity retained on the solid support.

A subject of the present invention is also the use of a molecule capable of binding to a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, and of inhibiting the tyrosine kinase activity of the insulin receptor, for manufacturing a medicinal product which can be used in the treatment of diseases involving insulin, in particular diabetes and obesity.

According to an advantageous embodiment of said use, said molecule is obtained using the method in accordance with the invention.

The compounds selected in this way can potentially be used for preventing or treating diseases involving insulin, such as for example diabetes and obesity or other pathological conditions characterized by insulin resistance, such as polycystic ovary syndrome (Legro, R.S. et al. (1998), *Rec. Progr. Hormone Res.*, **53**, 217-255) or syndrome X (Komers, R. et al., (1998), *Physiol. Res.*, **47**, 215-225).

Besides the arrangements above, the invention also comprises other arrangements which will emerge from the following description, which refers to examples of implementation of the method which is the subject of the present invention and also to the attached diagrams, in which:

- Figure 1 illustrates the alignment of the proteins of the family of Grb proteins. The percentages of amino acid identity of the domains are expressed relative to

the homologous domain of rGrb14. PP: motif rich in proline residues, binding site for proteins containing SH3 domains; PH: pleckstrin homology domain, association with phospholipids or proteins; PIR *phosphorylated insulin receptor interacting region*; SH2: domain allowing interaction with phosphotyrosine residues.

- Figure 2 illustrates the alignment of the sequences of the PIR domains of the proteins of the family of Grb proteins: rGrb14, hGrb14, hGrb10 and hGrb7. The numbering of the amino acids is given with reference to the sequence of the rGrb14 protein. The conserved amino acids are indicated with an asterisk. The conserved domain corresponding to residues 365-407 of the Grb proteins is in gray.

- Figure 3 illustrates the sequence of the PIR-SH2 domains of the proteins of the family of Grb proteins: rGrb14, hGrb10 and hGrb7. The complete sequence of the PIR-SH2 domain (residues 353-538) of rGrb14 (SEQ ID NO: 4), hGrb10 (SEQ ID NO: 16) and hGrb7 (SEQ ID NO: 24) is given. The sequence of fragment 405-538 of the PIR-SH2 domain of rGrb14 (SEQ ID NO: 3), hGrb10 (SEQ ID NO: 15) and hGrb7 (SEQ ID NO: 23) is underlined.

- Figure 4 illustrates the effect of the Grb proteins on the tyrosine kinase activity of insulin receptors; (●) GST-rGrb14; (▲) GST-mGrb10; (■) GST-rGrb7. The results are expressed as the percentage of the value obtained in the absence of added protein. Number of experiments = 4. The effects of the GST-mGrb10 and GT-rGrb7 proteins compared with those of the GST-rGrb14 protein show statistically significant differences, indicated by * for $p<0.05$, ** for $p<0.01$ and *** for $p<0.001$.

- Figure 5 illustrates the inhibition of the tyrosine kinase activity of insulin receptors by the rGrb14 protein: (●) GST-rGrb14; (▲) GST-PIR of rGrb14; (☒) GST-SH2 of rGrb14; (■) GST-PIR+SH2 of rGrb14; (◊) GST-PIR+SH2 R464K of rGrb14. The results are expressed as the percentage of the value obtained in the absence of added protein. Number of experiments = 5. The effects of the various rGrb14 constructs compared with those of the whole GST-rGrb14 protein show statistically significant differences, indicated by * for $p<0.05$, ** for $p<0.01$ and *** for $p<0.001$.

- Figure 6 illustrates the inhibition of the tyrosine kinase activity of insulin receptors by the various domains of mGrb10: (●) GST-mGrb10; (▲) GST-PIR of mGrb10; (☒) GST-SH2 of mGrb10; (■) GST-PIR+SH2 of mGrb10; (◊) GST-PIR+SH2 R547K of mGrb10. The results are expressed as a percentage of the value obtained in the absence of added protein. Number of experiments = 4. The effects of the various mGrb10 constructs compared with those of the whole GST-mGrb10 protein show statistically significant differences, indicated by: * for $p<0.05$, ** for $p<0.01$ and *** for $p<0.001$.

- Figure 7 illustrates the inhibition of the tyrosine kinase activity of insulin receptors by the various domains of rGrb7: (●) GST-rGb7; (▲) GST-PIR of rGb7; (☒) GST-SH2 of rGrb7; (■) GST-PIR+SH2 of rGrb7. The results are expressed as a percentage of the value obtained in the absence of added protein. Number of experiments = 2. The effects of the various rGrb7 constructs compared with those of the whole GST-rGrb7 protein show statistically significant differences, indicated by: * for $p<0.05$, ** for $p<0.01$ and *** for $p<0.001$.

Example 1: Comparison of the effect of the rGrb14, mGrb10 and rGrb7 proteins on the tyrosine kinase activity of insulin receptors

1. Procedure:

Insulin receptors are partially purified from CHO-IR cells by passing a cell lysate over a weak germ lectin column and eluting the glycoproteins retained with 0.3 M N-acetylglucosamine. The insulin receptors thus purified are incubated in the presence of insulin (0 or 10^{-7} M) for 1 hour at room temperature. A buffer containing 20 µM ATP, $MnCl_2$ and $MgCl_2$ ions and [γ-^{32}P] ATP is then added so as to allow the receptors to autophosphorylate, as are increasing amounts of the purified Grb proteins expressed as a fusion with GST. 30 minutes later, 15 µg of a synthetic substrate, poly Glu-Tyr (4:1), are added. The tyrosine kinase activity of the receptors is measured by the incorporation of radioactivity into the poly Glu-Tyr during 30 min.

2. Results:

They are represented in Figure 4.

The addition of the GST-rGrb14 and GST-mGrb10 fusion proteins induces dose-dependent inhibition of the tyrosine kinase activity of the insulin receptors, and the highest concentrations allow total inhibition of the enzyme. By comparison, the GST-rGrb7 protein enables a maximum of only 40% inhibition. The dose-response curve of the effect of GST-mGrb10 is shifted to the right compared to the curve of the effect of GST-rGrb14. 50% inhibition of the tyrosine kinase activity of the receptors is obtained when using, respectively, 0.04 µg of GST-rGrb14 and 0.13 µg of GST-mGrb10. The tyrosine kinase activity of the insulin

receptors is therefore more sensitive to the inhibitory effect of rGrb14 than to that of mGrb10.

These results show that the Grb proteins have inhibitory activity on the tyrosine kinase activity of insulin receptors and that the rGrb14 protein has the greatest inhibitory effect.

Example 2: Inhibitory effect of the various domains of rGrb14 on the tyrosine kinase activity of insulin receptors

1. Procedure:

The insulin receptors are partially purified as described in Example 1. The various domains of rGrb14 (rGrb14, PIR, SH2, PIR+SH2, PIR+SH2 R464K) are produced as a fusion with GST and purified. The inhibitory effect of these proteins on the tyrosine kinase activity of insulin receptors is analyzed as described in Example 1.

2. Results:

They are as represented in Figure 5.

The PIR domain exerts an inhibitory effect comparable to that of the whole rGrb14 protein, whereas the SH2 domain has no effect (in the same way as the protein deleted of the PIR+SH2 regions, results not shown). However, the PIR+SH2 domain has a much greater inhibitory effect than PIR alone or the whole protein (the maximum inhibitory effect is obtained by adding 0.03 µg of protein). This potentiation is suppressed by mutating the arginine 464 residue of the conserved FLVRES motif, which inactivates the SH2 domain, since the PIR+SH2 R464K domain has the same effect as PIR alone.

These results show that the inhibitory effect of rGrb14 on the tyrosine kinase activity of insulin receptors is due to the presence of PIR. The SH2 domain alone has no effect, but it potentiates the PIR effect.

Example 3: Inhibitory effect of the various domains of mGrb10 on the tyrosine kinase activity of insulin receptors

1. Procedure:

The procedure is identical to that described in Example 2.

2. Results:

They are represented in Figure 6.

The PIR domain exerts an inhibitory effect comparable to that exerted by the whole protein. The SH2 domain alone has no inhibitory action, but it potentiates the inhibition exerted by PIR (the dose-response curve of PIR+SH2 is shifted to the left). Mutating the arginine residue of the FLLRDS motif of the SH2 domain inhibits the potentiating effect of the PIR+SH2 domain (PIR+SH2 R547K mutant).

These results show that the PIR or PIR-SH2 domains of rGrb14 have a greater inhibitory effect than the PIR or PIR-SH2 domains of mGrb10.

Example 4: Inhibitory effect of the various domains of rGrb7 on the tyrosine kinase activity of the insulin receptors

1. Procedure:

The procedure is identical to that described in Example 2.

2. <u>Results</u>:

They are represented in Figure 7.

The PIR domain exerts an inhibitory effect comparable to that exerted by the whole protein. The SH2 domain alone has no inhibitory action, but it potentiates the inhibition exerted by PIR (the dose-response curve of PIR+SH2 is shifted to the left). The PIR and PIR-SH2 domains of rGrb7 have a lesser inhibitory effect compared to that of the PIR and PIR-SH2 domains of the rGrb14 and mGrb10 proteins.

As emerges from the above, the invention is in no way limited to its methods of implementation, preparation and application which have just been described more explicitly; on the contrary, it encompasses all of the variants thereof which may occur to a person skilled in the art, without departing from the context or scope of the present invention.

CLAIMS

1. The use of a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, as a tool for screening for molecules intended for treating diseases involving insulin.

2. The use as claimed in claim 1, characterized in that said fragment is selected from the group consisting of the sequences SEQ ID NO: 1-28.

3. A method for detecting molecules capable of modulating the tyrosine kinase activity of the insulin receptor, characterized in that it comprises:

a) bringing the activated insulin receptor into contact with a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, and the molecule to be tested, under conditions which allow binding of said fragment to said receptor,

b) adding a tyrosine kinase substrate,

c) measuring the tyrosine kinase activity, and

d) determining the modulation of the tyrosine kinase activity by comparison with a control consisting of the activated insulin receptor and said fragment.

4. The method as claimed in claim 3, characterized in that said fragment is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 28.

5. The method as claimed in claim 3 or claim 4, characterized in that, prior to step a), a preselection of the molecules capable of modulating the interactions of a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, with the insulin receptor, is carried out by:

1) immobilizing said fragment on a solid support,

2) bringing the molecule to be tested into contact with said fragment, then

3) incubating with the labeled and pre-activated insulin receptor, under conditions which allow binding of said receptor to said fragment,

4) separating said labeled receptor not retained on the support,

5) detecting the complex possibly formed between said fragment and the activated insulin receptor, and

6) determining the effect of the molecule by comparison with a control comprising said fragment and the insulin receptor.

6. The use of a molecule capable of binding to a fragment consisting of the PIR domain or the PIR-SH2 domain of a protein of the family of Grb7 proteins, and of inhibiting the tyrosine kinase activity of the insulin receptor, for manufacturing a medicinal product which can be used in the treatment of diseases involving insulin.

7. The use as claimed in claim 6, characterized in that said molecule is obtained using the method as claimed in any one of claims 3 to 5.

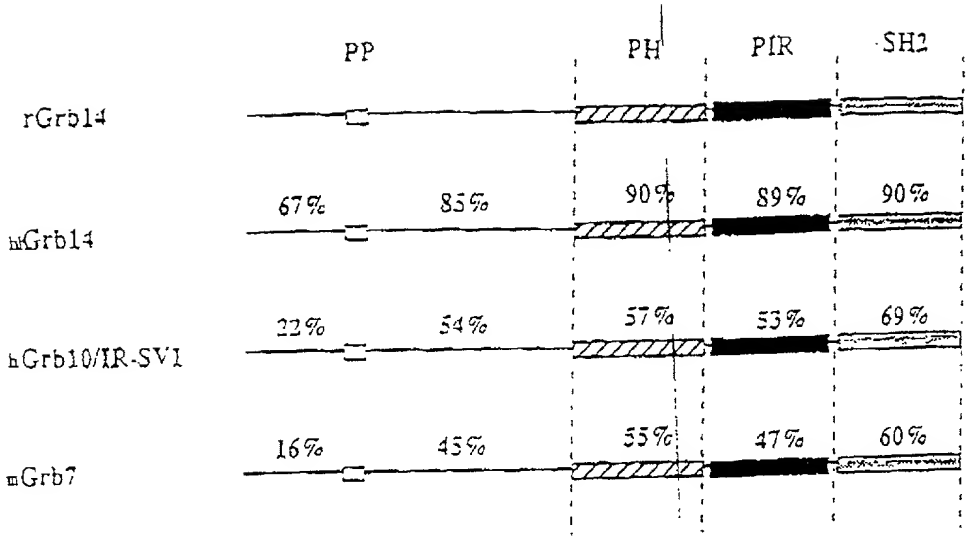
PP
PH
PIR
SH2
rGrb14
hGrb14
67%
85%
90%
89%
90%
hGrb10/IR-SV1
22%
54%
57%
53%
69%
mGrb7
16%
45%
55%
47%
60%

FIG. 1

09/936697

2/7

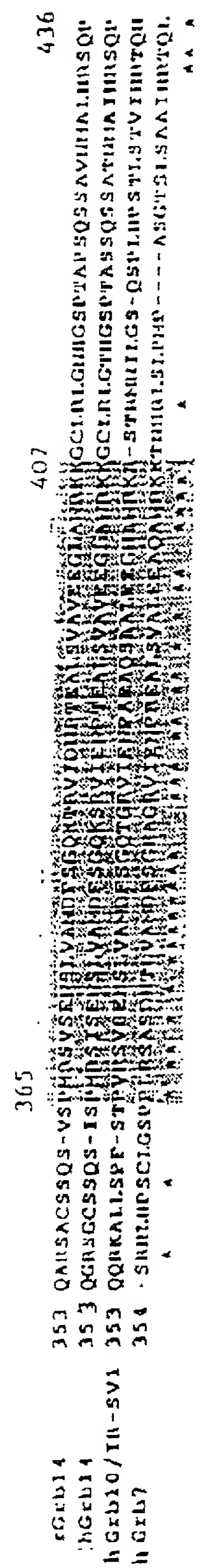

FIG. 2

PIR-SH2 domains:

rGrb14

QARSACSSQSVSPMRSVSENSLVAMDFSGQKTRVIDNPTEALSVAVEEGLAWRKKGC
LRLGNHGSPTAPSQSSAVNMALHRSQPWFHHRISRDEAQQLITRQGPVDGVFLVRDS
QSNPRTFVLSMSHGQKIKHFQIIPVEDDGEVFHTLDDGHTKFTDLIQLVEFYQLNKG
VLPCKLKHYCARMAV

hGrb10

QQRKALLSPFSTPVRSVSENSLVAMDFSGQTGRVIENPAEAQSAALEEGHAWRKRST
RMNILGSQSPLHPSTLSTVIHRTQHWFHGRFSREESHRIIKQQGLVDGLFLLRDSQS
NPKAFVLTLCHHQKIKNFQILPCEDDGQTFFSLDDGNTKFSDLIQLVDFYQLNKGVL
PCKLKHHCIRVAL

hGrb7

SRHLHPSCLGSPPLRSASDNTLVAMDFSGHAGRVIENPREALSVALEEAQAWRKKTN
HRLSLPMPASGTSLSAAIHRTQLWFHGRISREESQRLIGQQGLVDGLFLVRESQRNP
QGFVLSLCHLQKVKHYLILPSEEEGRLYFSMDDGQTRFTDLLQLVEFHQLNRGICLL
RHCCTRVAL

FIGURE 3

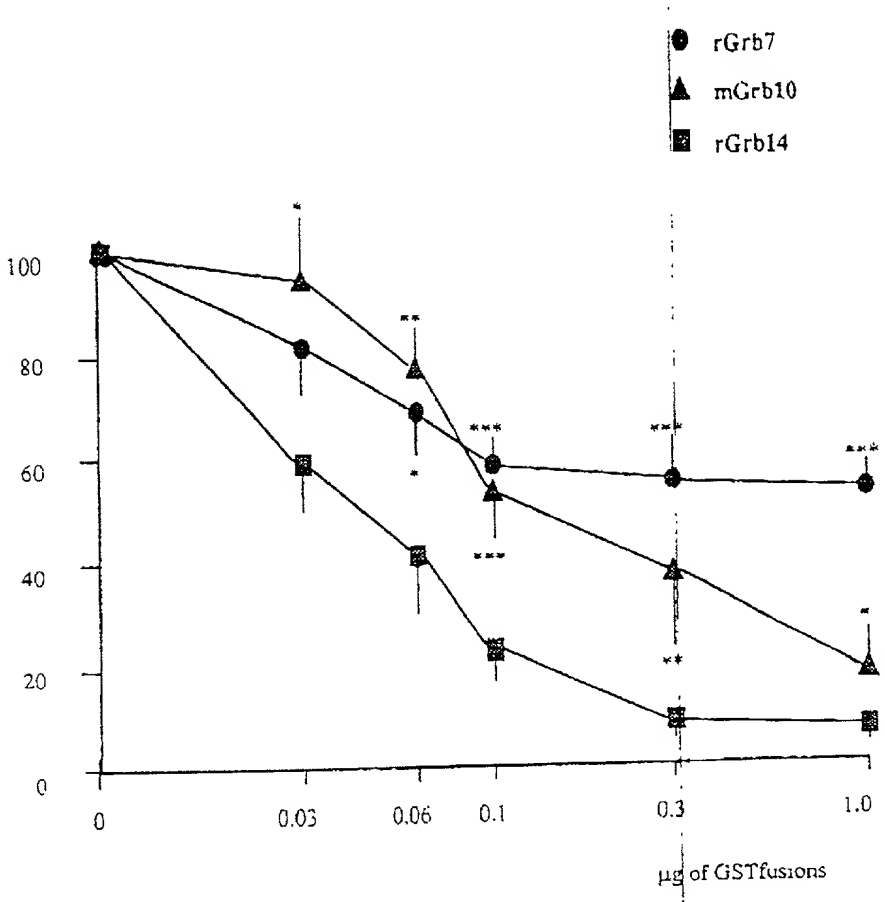
rGrb7
mGrb10
rGrb14
100
80
60
40
20
0
0
0.03
0.06
0.1
0.3
1.0
µg of GSTfusions

Figure 4

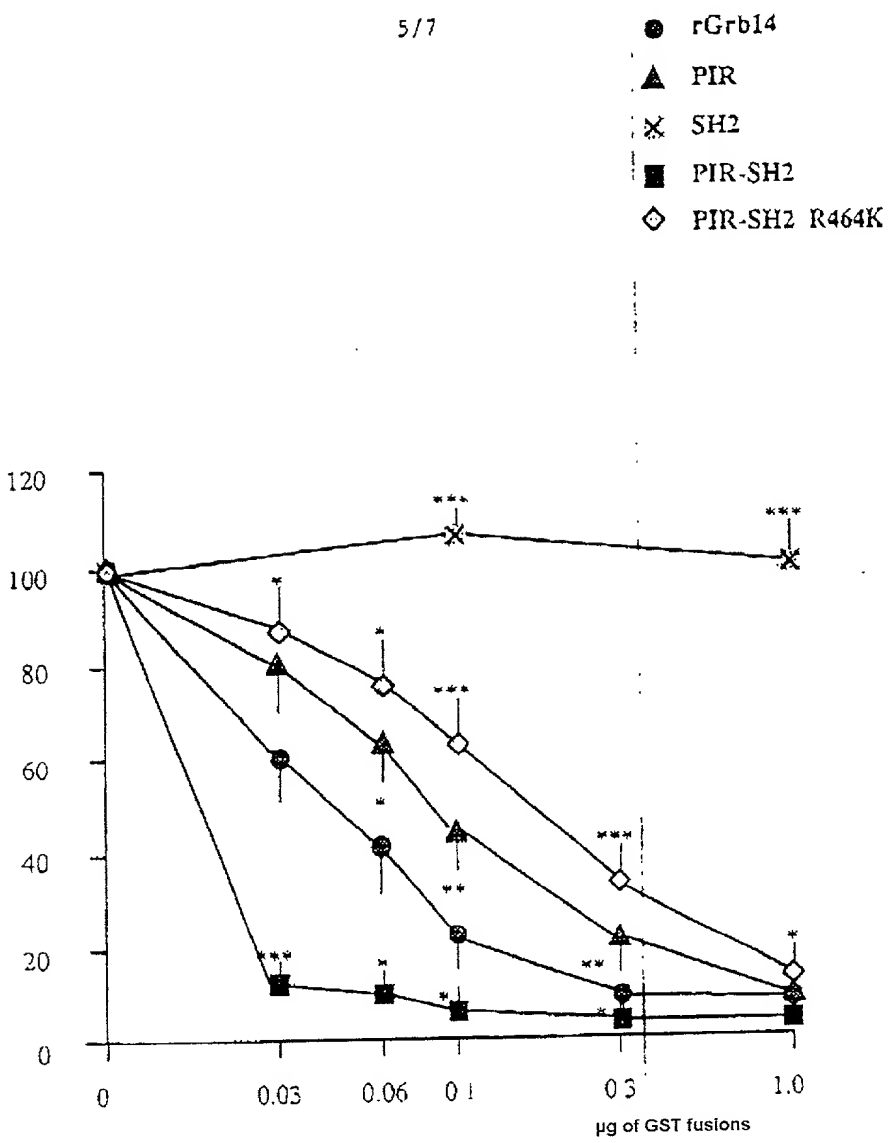
rGrb14
PIR
SH2
PIR-SH2
PIR-SH2 R464K
120
100
80
60
40
20
0
0
0.03
0.06
0.1
0.3
1.0
µg of GST fusions

Figure 5

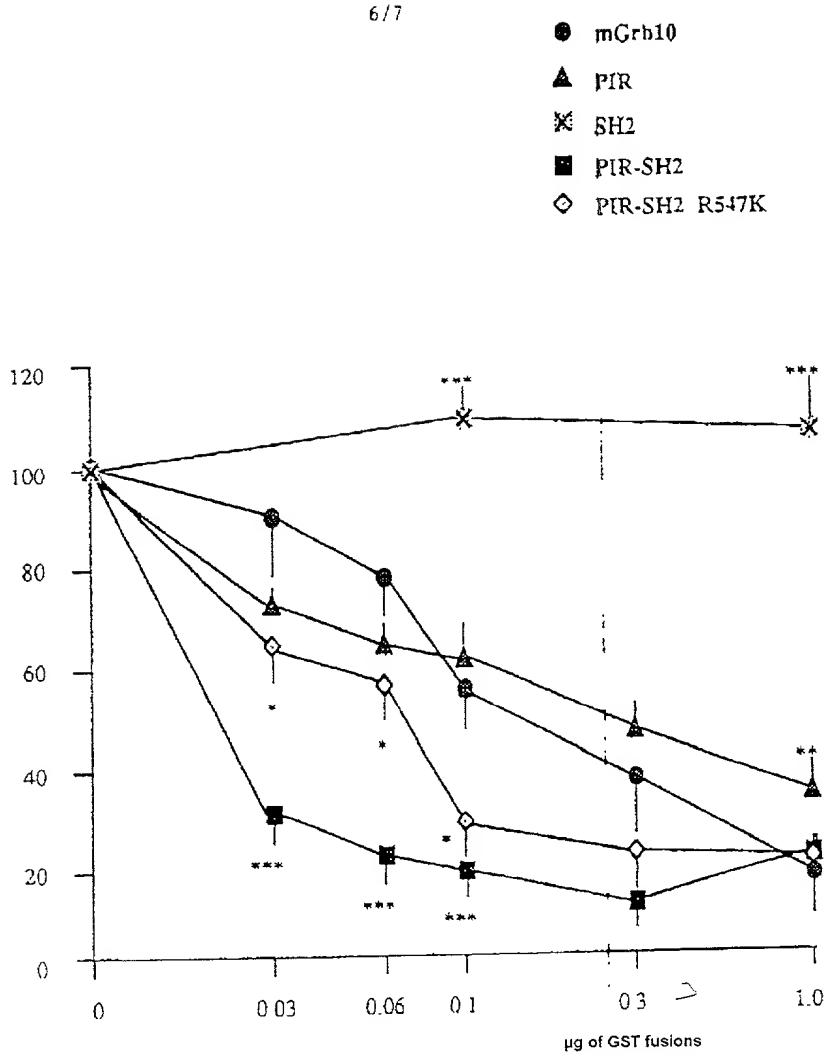
mGrb10
PIR
SH2
PIR-SH2
PIR-SH2 R547K
120
100
80
60
40
20
0
0
0 03
0.06
0 1
0 3
1.0
µg of GST fusions

Figure 6

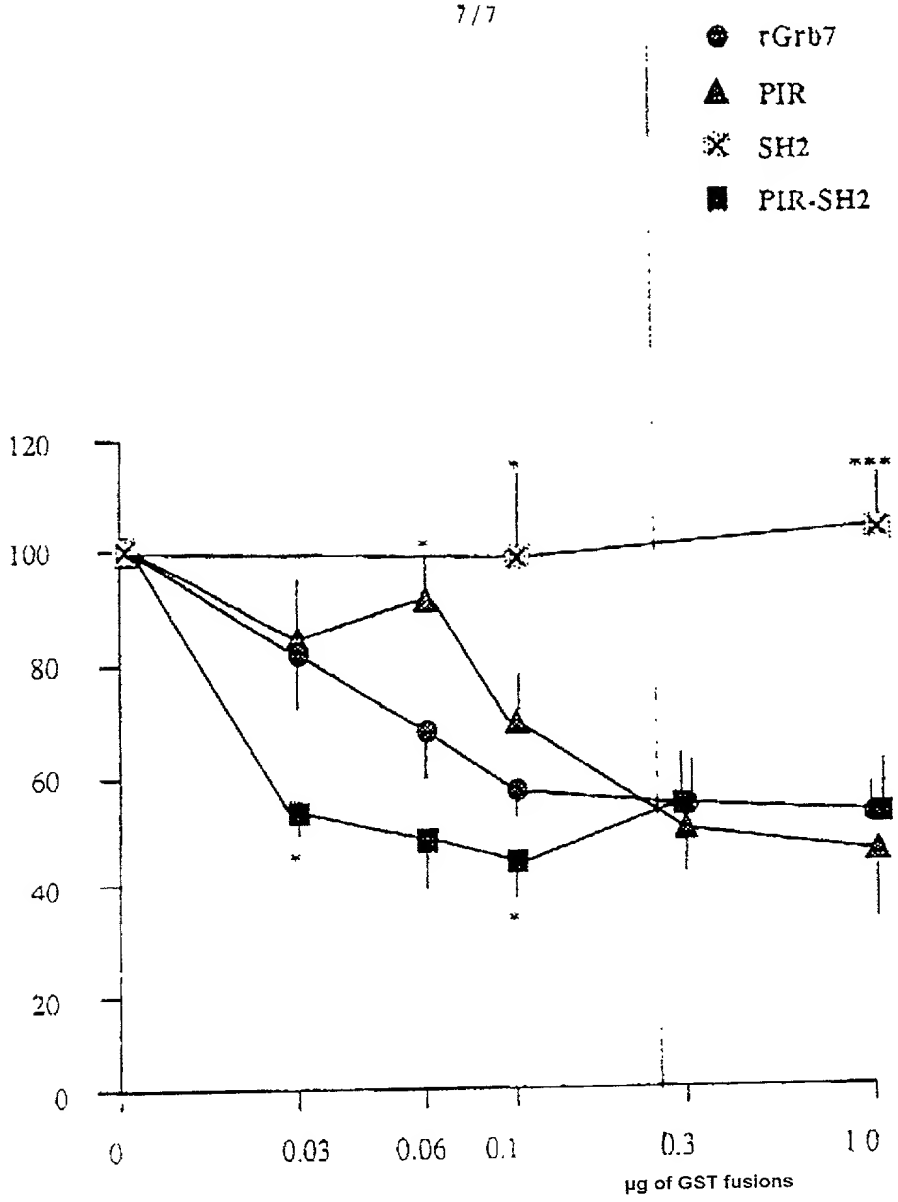
rGrb7
PIR
SH2
PIR-SH2
120
100
80
60
40
20
0
0
0.03
0.06
0.1
0.3
1 0
µg of GST fusions
***

Figure 7

DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

ATTORNEY DOCKET NO. :

As a below named inventor, I hereby declare that :

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled :

GRB 14 AND THE INSULIN RECEPTOR AND SCREENING OF NOVEL MEDICINES

the specification of which :

is attached hereto ; or

was filed as United States application Serial No. _____ on _____ and was amended on _____ (if applicable) ; or

was filed as a PCT international application Number PCT/FR00/00613 on March 14, 2000 and was amended under PCT article 19 on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose the U.S. Patent and Trademark Office information which is material to the patentability of claims presented in this application in accordance with Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed :

PRIOR FOREIGN APPLICATION(S) :

COUNTRY (if PCT, indicate PCT)	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
FRANCE	99/03159	15 March 1999	X

Combined Declaration For Patent Application and Power of Attorney – **(Continued)**
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NO. :

I hereby claim the benefits under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

U.S. PROVISIONAL APPLICATIONS

U.S. PROVISIONAL APPLICATION NO.	U.S. FILING DATE

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or §365 (c) of any PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the U.S Patent and Trademark Office all information known to me to be material to the patentability of claims represented in this application in accordance with Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application :

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT :

U.S. APPLICATIONS		STATUS (Check one)		
U.S. APPLICATION NO.	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the registered practitioners of Morgan, Lewis & Bockius LLP included in the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct all correspondence be addressed to that Customer Number.

Customer Number : 009629

Direct Telephone Calls To :
(name and telephone number)

Combined Declaration For Patent Application and Power of Attorney – **(Continued)**
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NO. :

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	**BURNOL Anne-Françoise**	
RESIDENCE & CITIZENSHIP	SEVRES, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	52, Grande Rue 92310 SEVRES FRANCE	
FIRST OR SOLE INVENTOR'S SIGNATURE X		DATE
FULL NAME OF SECOND INVENTOR	**PERDEREAU Dominique**	
RESIDENCE & CITIZENSHIP	ISSY LES MOULINEAUX, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	16, Rue Henri Tariel 92130 ISSY LES MOULINEAUX FRANCE	
SECOND INVENTOR'S SIGNATURE X D. Perdereau PRX		DATE 25 JUIN 01
FULL NAME OF THIRD INVENTOR	**KASUS-JACOBI Anne**	
RESIDENCE & CITIZENSHIP	MONTIGNY LE BRETONNEUX, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	5 Place Louis Durey 78180 MONTIGNY LE BRETONNEUX FRANCE	
THIRD INVENTOR'S SIGNATURE		DATE
FULL NAME OF FOURTH INVENTOR	**BEREZIAT Véronique**	
RESIDENCE & CITIZENSHIP	PALAISEAU, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	24, Rue du Hameau des Joncherettes 91120 PALAISEAU FRANCE	
FOURTH INVENTOR'S SIGNATURE		DATE

Listing of Inventors Continued on attached page(s) [X] Yes [ ] No

Combined Declaration For Patent Application and Power of Attorney – **(Continued)**
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NO. :

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	**BURNOL Anne-Françoise**	
RESIDENCE & CITIZENSHIP	SEVRES, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	52, Grande Rue 92310 SEVRES FRANCE	
FIRST OR SOLE INVENTOR'S SIGNATURE		DATE
FULL NAME OF SECOND INVENTOR	**PERDEREAU Dominique**	
RESIDENCE & CITIZENSHIP	ISSY LES MOULINEAUX, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	16, Rue Henri Tariel 92130 ISSY LES MOULINEAUX FRANCE	
SECOND INVENTOR'S SIGNATURE		DATE
FULL NAME OF THIRD INVENTOR	**KASUS-JACOBI Anne**	
RESIDENCE & CITIZENSHIP	MONTIGNY LE BRETONNEUX, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	5 Place Louis Durcy 78180 MONTIGNY LE BRETONNEUX FRANCE	
THIRD INVENTOR'S SIGNATURE	A.Kasus	DATE 24/08/2001
FULL NAME OF FOURTH INVENTOR	**BEREZIAT Véronique**	
RESIDENCE & CITIZENSHIP	PALAISEAU, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	24, Rue du Hameau des Joncherettes 91120 PALAISEAU FRANCE	
FOURTH INVENTOR'S SIGNATURE		DATE

Listing of Inventors Continued on attached page(s) [X] Yes [ ] No

Combined Declaration For Patent Application and Power of Attorney – **(Continued)**
(includes Reference to PCT International Applications)

ATTORNEY DOCKET NO. :

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

FULL NAME OF SOLE OR FIRST INVENTOR	**BURNOL Anne-Françoise**	
RESIDENCE & CITIZENSHIP	SEVRES, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	52, Grande Rue 92310 SEVRES FRANCE	
FIRST OR SOLE INVENTOR'S SIGNATURE		DATE 24/08/01
FULL NAME OF SECOND INVENTOR	**PERDEREAU Dominique**	
RESIDENCE & CITIZENSHIP	ISSY LES MOULINEAUX, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	16, Rue Henri Tariel 92130 ISSY LES MOULINEAUX FRANCE	
SECOND INVENTOR'S SIGNATURE		DATE
FULL NAME OF THIRD INVENTOR	**KASUS-JACOBI Anne**	
RESIDENCE & CITIZENSHIP	MONTIGNY LE BRETONNEUX, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	5 Place Louis Durey 78180 MONTIGNY LE BRETONNEUX FRANCE	
THIRD INVENTOR'S SIGNATURE		DATE
FULL NAME OF FOURTH INVENTOR	**BEREZIAT Véronique**	
RESIDENCE & CITIZENSHIP	PALAISEAU, FRENCH	COUNTRY OF CITIZENSHIP FRANCE
POST OFFICE ADDRESS	24, Rue du Hameau des Joncherettes 91120 PALAISEAU FRANCE	
FOURTH INVENTOR'S SIGNATURE		DATE 24/08/01

Listing of Inventors Continued on attached page(s) [X] Yes [ ] No

FULL NAME OF FIFTH INVENTOR	GIRARD Jean 5-00		
RESIDENCE & CITIZENSHIP	PARIS , FRENCH	COUNTRY OF CITIZENSHIP FRANCE	
POST OFFICE ADDRESS	54, Rue Vergniaud 75013 PARIS FRANCE FRX		
FIFTH INVENTOR'S SIGNATURE		DATE	24/08/01
FULL NAME OF SIXTH INVENTOR			
RESIDENCE & CITIZENSHIP		COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS			
SIXTH INVENTOR'S SIGNATURE		DATE	
FULL NAME OF SEVENTH INVENTOR			
RESIDENCE & CITIZENSHIP		COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS			
SEVENTH INVENTOR'S SIGNATURE		DATE	
FULL NAME OF EIGHTH INVENTOR			
RESIDENCE & CITIZENSHIP		COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS			
EIGHTH INVENTOR'S SIGNATURE		DATE	
FULL NAME OF NINTH INVENTOR			
RESIDENCE & CITIZENSHIP		COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS			
NINTH INVENTOR'S SIGNATURE		DATE	